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TWO-LASER MATRIX ASSISTED LASER DESORPTION/LASER IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY OF BIOMOLECULES

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Recent efforts to detect and identify large biomolecules using matrix assisted laser desorption have shown MALDI to be an extremely sensitive technique for trace detection from a solid sample. In the MALDI process, ions are formed by proton transfer from an electronically excited organic acid to a suitable proton accepting analyte. However, detection and characterization of biomolecules may benefit from ionization of the isolated unprotonated gas phase neutrals. To this end, a custom time-of-flight mass spectrometer has been constructed to perform two-laser matrix assisted laser desorption/laser ionization studies using separate desorption and ionization lasers. The two chamber, differentially pumped apparatus incorporates numerous laser and sample probe entry ports, a pulsed valve, and a modified Wiley-McLaren mass spectrometer design. Results of initial two-laser experiments on phenylalanine in a sinnapinic acid matrix have demonstrated the apparatus' ability to detect laser ionization of laser desorbed neutrals.

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PREFACE

The work described in this report was authorized under Contract No. DAAA15-93-C-0021. This work was started in July 1993 and completed in October 1995.

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TWO-LASER MATRIX ASSISTED LASER DESORPTION/LASER IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY OF BIOMOLECULES

INTRODUCTION

Matrix assisted laser desorption/ionization is one of the promising techniques which may lead to efficient detection and identification of biomolecules. As such, considerable effort has been and is cuurently being expended to develop new matrices, identify optimum desorption/ionization conditions, and increase experimental detection efficiency and resolution. In addition alternative approaches are being explored, including two-laser experiments wherein the desorption event is spatially and temporally separated from the ionization event. Since laser conditions conducive to efficient desorption may be vastly different from those appropriate for efficient ionization, separation of the two processes may yield better results (higher sensitivity, better resolution, better mass accuracy) than the single laser MALDI experiment.

To this end we have developed, constructed, and tested a custom mass spectrometer to perform two-step laser desorption/ laser ionization time-of-flight mass spectrometry of biomolecules. The instrument design permits desorption and/or ionization at either the output of a pulsed valve or in the ionization region of the mass spectrometer. In addition, the sample probe may be oriented in several configurations relative to the desorption and ionization lasers. Finally, entrainment of the ionized sample in a pulsed gas beam has been incorporated in the design of the instrument.

EXPERIMENTATION

The apparatus assembled to perform the two-laser MALDI experiments is a differentially pumped two chamber vacuum system. Laser entrance and exit ports and sample probe inlet ports are incorporated in both the lower "source" chamber (figure 1) and in the upper mass spec chamber (figure 2). The time-of-flight mass spectrometer ion optics, located in the upper mass spec chamber, are shown in figure 3. Not shown in these figures are the numerous control and data acquisition electronics required by these experiments (delay generators, boxcar integrators, computer interface module, laser power meters, stepper motor controllers, digital storage oscilloscope). During an experiment many of these devices are controlled via computer using software developed in-house. In addition, the data reduction and analysis routines were all developed in-house.

The lasers utilized in these experiments were a SpectraPhysics DCR-2A and a Continuum PY61C. Both lasers were nanosecond pulsed lasers with associated dye lasers and doubling crystals for extension of the tunable dye laser output into the ultraviolet. Both lasers had three signal inputs for external control and/or synchronization.

In order to perform the two-laser desorption/ionization experiments a custom electronics module was designed and built to synchronize two Nd:YAG lasers and a pulsed valve. The module supplied three triggers of varying pulsewidths and pulseheights to each of the lasers and a TTL level trigger to the pulsed valve. The module operated at repetition rates of .5 Hz to 10 Hz. In addition, the module could be externally triggered to operate in a single-shot mode. An electronic diagram of the module is shown in figures 4 and 5.

RESULTS

Numerous experiments were performed to test and calibrate the instrument prior to the two-laser MALDI experiments. The first set of experiments involved synchronization of the pulsed molecular beam with the ionization laser. This was accomplished as follows. The carbon dioxide pulsed valve gas was bubbled through methyl iodide (liquid) in route to the pulsed valve. The resultant pulsed gas beam contained several percent methyl iodide. The laser "oscillator out" sync pulse was used to trigger a delay generator, the output of which then triggered the pulsed valve. The fourth harmonic (266 nm) of the Nd:YAG was focused into the center of the ionization region of the mass spectrometer. By maximizing the intensity of the I^+ signal which results from dissociation/ionization of methyl iodide at 266 nm the ionizing laser pulse and gas pulse synchronization was optimized. A representative mass spectrum obtained in these experiments is shown in figure 6.

The second set of experiments involved calibration of the dye laser wavelength for tunable ionization experiments. In these experiments pure krypton was used as the pulsed valve gas. The dye laser was pumped by the second harmonic (532 nm) of the Nd:YAG laser. The visible light pulse was frequency doubled using doubling crystals in an autotracking wavelength extender (WEX). The resultant tunable UV light pulses were focused into the ionization region of the mass spectrometer. By recording the intensity of the krypton ion while scanning the dye laser wavelength, ion signals were observed which correspond to three-photon resonances in atomic krypton. The dye laser wavelength scale was calibrated by comparing the observed energies of these resonances with their literature values. Representative data for these experiments is shown in figure 7.

In the two-laser desorption/ionization experiments $\sim 300\mu$l of a .01M sinnapinic acid/methanol solution was deposited on the probe tip. Before it could dry $\sim 100\mu$l of a .001M phenylalanine/methanol solution was deposited on the probe tip as well. The probe was allowed to air dry. The probe was then inserted into the mass spectrometer as shown in figure 2. The probe tip was positioned approximately 2.5 cm from the center of the ionization region of the mass spectrometer. The desorption laser (355 nm, $\sim 150\mu$J) was focused onto the probe tip with a 350 mm focal length lens. The ionization laser (266 nm, $\sim 200\mu$J) was delayed by $\sim 30\mu$s relative to the desorption laser. The resultant mass spectrum (figure 8) displays signals due to laser ionization and fragmentation of the phenylalanine analyte and sinnapinic acid matrix.

Signal levels acquired in these experiments appear comparable to those observed in single laser MALDI experiments. This result in encouraging in that only a small fraction of the desorbed neutral plume is interrogated and ionized by the second laser in the two-laser experiment. This confirms earlier work suggesting that the large majority of species in the desorption plume are neutrals rather than ions. As such, more efficient ionization of the desorption plume should enhance signal levels and therefore detection sensitivities.

The resolution observed in the present two-laser experiments appears comparable to that observed in the single laser MALDI experiment at the low masses studied to this point. The resolution observed in time-of-flight mass spec experiments depends predominately on two factors, the intial ion spatial distribution and the initial ion kinetic energy spread. As long as thin samples and low laser powers are utilized, resolution in the single laser MALDI experiment does not suffer greatly from spatial spread. In the two-laser experiment the initial ion spatial distribution is determined by the ionization laser spot size. Dual stage ion extraction can largely eliminate the deterioration of resolution in the two-laser experiment.

The kinetic energy spread observed in the single laser MALDI experiment is largely circumvented by utilizing high extraction fields in the source region. Although the desorbed neutrals in the two-laser experiment also possess a large kinetic energy spread, this spread is in the direction perpendicular to the ion collection axis. Hence, as long as these ions can be steered onto the detector using deflection plates the large initial kinetic energy spread does not significantly deteriorate resolution. In addition, various schemes exist to improve resolution due to initial kinetic energy spread in the two-laser experiment (time-lag focussing, post-source pulse-focussing (PSPF)). The fourth ion acceleration grid required to perform post-source pulse-focussing is incorporated in the present apparatus (see figure 3). However, because the technique is primarily useful for high-mass ions, it has not yet been implemented.

The mass accuracy of the two-laser experiments is difficult to compare to the single laser experiments with the limited data available at this time. The mass determination routines used to analyze the existing data for the two-laser experiment (figure 9) result in mass accuracies of +/- 0.5 amu at masses above 50 amu. This level of accuracy is comparable to that observed in single-color gas phase laser photoionization experiments using similar calibration procedures. Depending on experimental conditions and mass range, the multipoint calibration algorithms included with many commercial single laser MALDI systems typically exhibit mass accuracies of a few tenths of an amu. The lack of mass accuracy of the two laser experiment can be attributed to experimental uncertainties in the ionization volume location, distortion of electric field lines in the ionization region due to the presence of the sample probe, and inadequacies of the fitting algorithms used in fitting the two color data. Other as yet undetermined experimental factors may also contribute.

CONCLUSIONS

A custom two-laser matrix assisted laser desorption/laser ionization time-of-flight mass spectrometer has been designed, constructed, and successfully demonstrated in detecting gas phase biomolecular ions from an organic acid solid matrix.

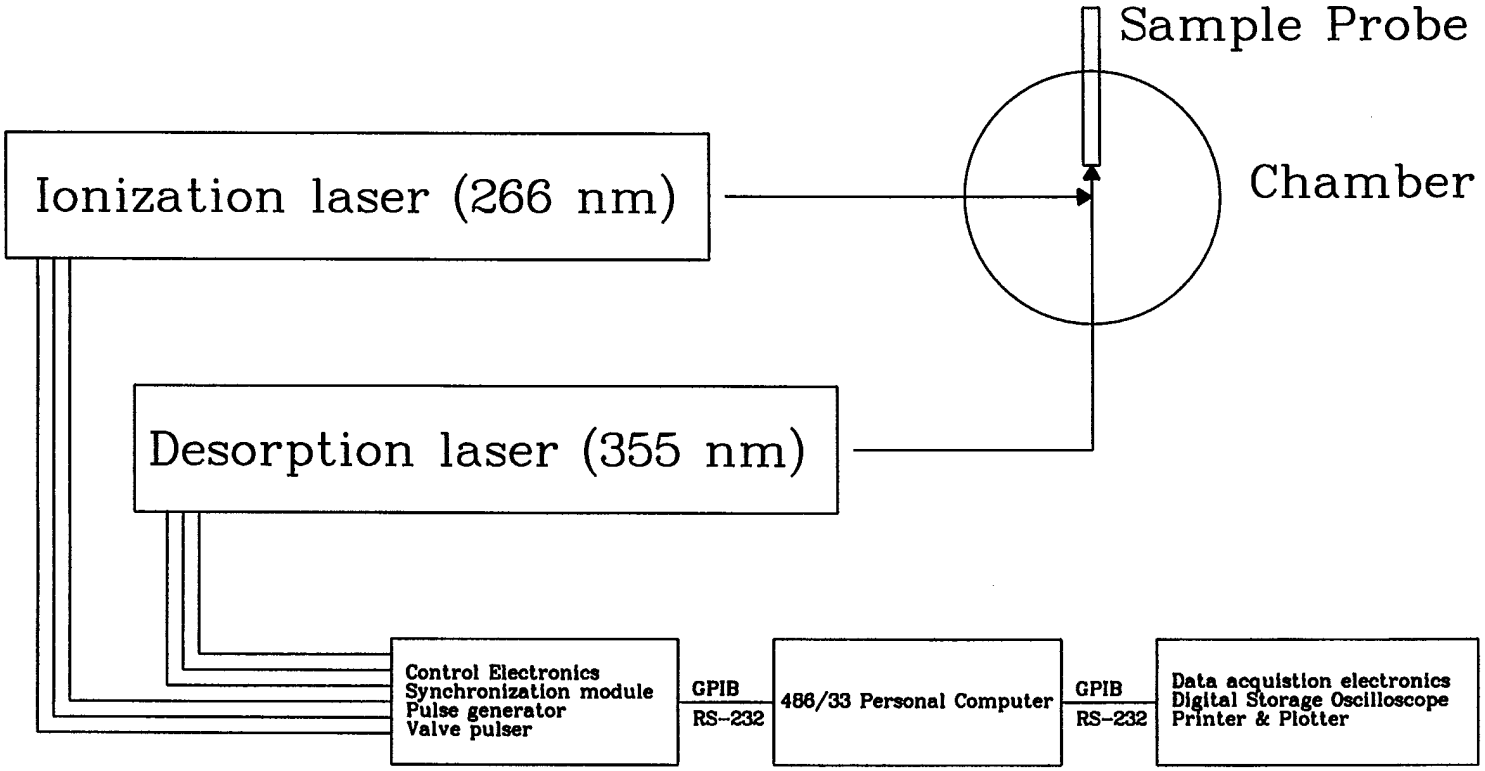

Figure 1: LD/LI TOF-MS Experimental setup diagram. Each of the lasers is supplied three synchronization pulses by a custom NIM module (figures 5 and 6). A pulsed valve receives a separate synchronization pulse. Structural determination of the ions formed by LD/LI is achieved using pulsed extraction followed by time-of-flight mass analysis and microchannel plate detection.

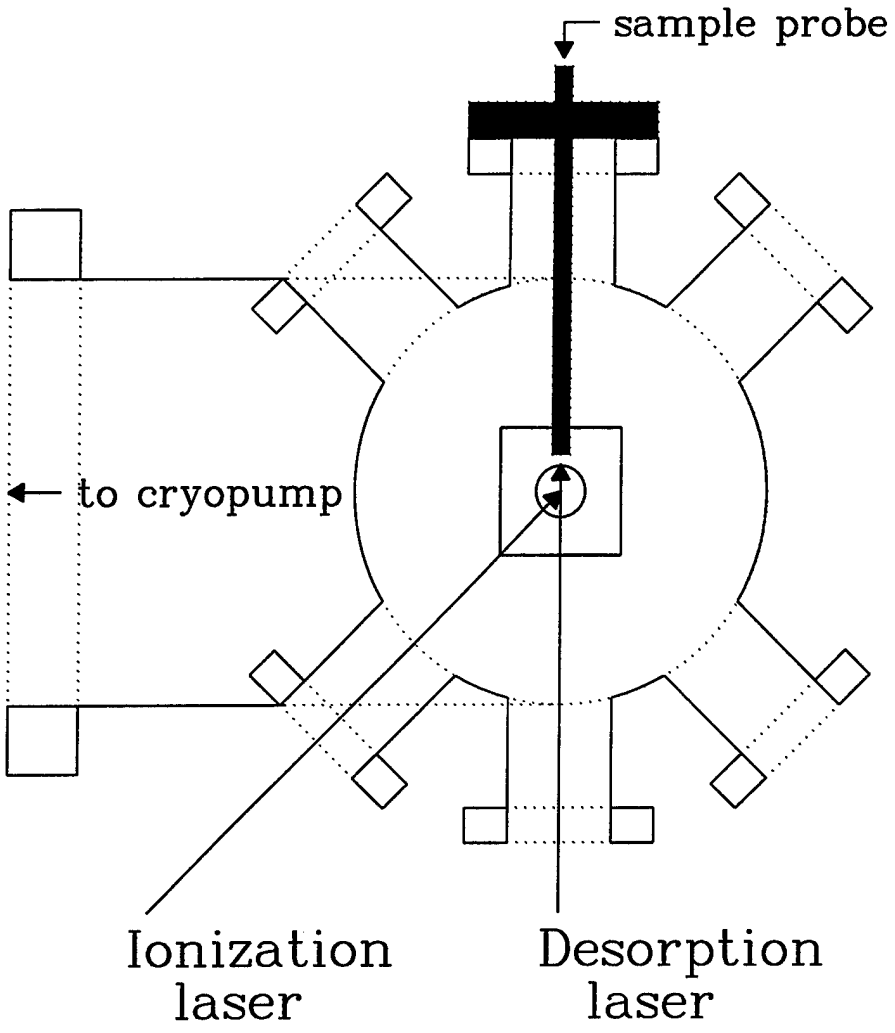

Figure 2: Top view of the LD/LI TOF–MS source chamber. In the configuration shown, the probe is positioned directly above and slightly off of the pulsed valve axis. Alternatively, the ionization laser can cross the gas beam after it has passed into the ionization region of the mass spectrometer. The sample probe may be inserted through any of the available ports. Neutrals produced by the desorption laser pulse are entrained by the gas pulse and transported into the upper mass spec chamber of the apparatus for ionization by the second laser, followed by time–of–flight mass separation and microchannel plate detection.

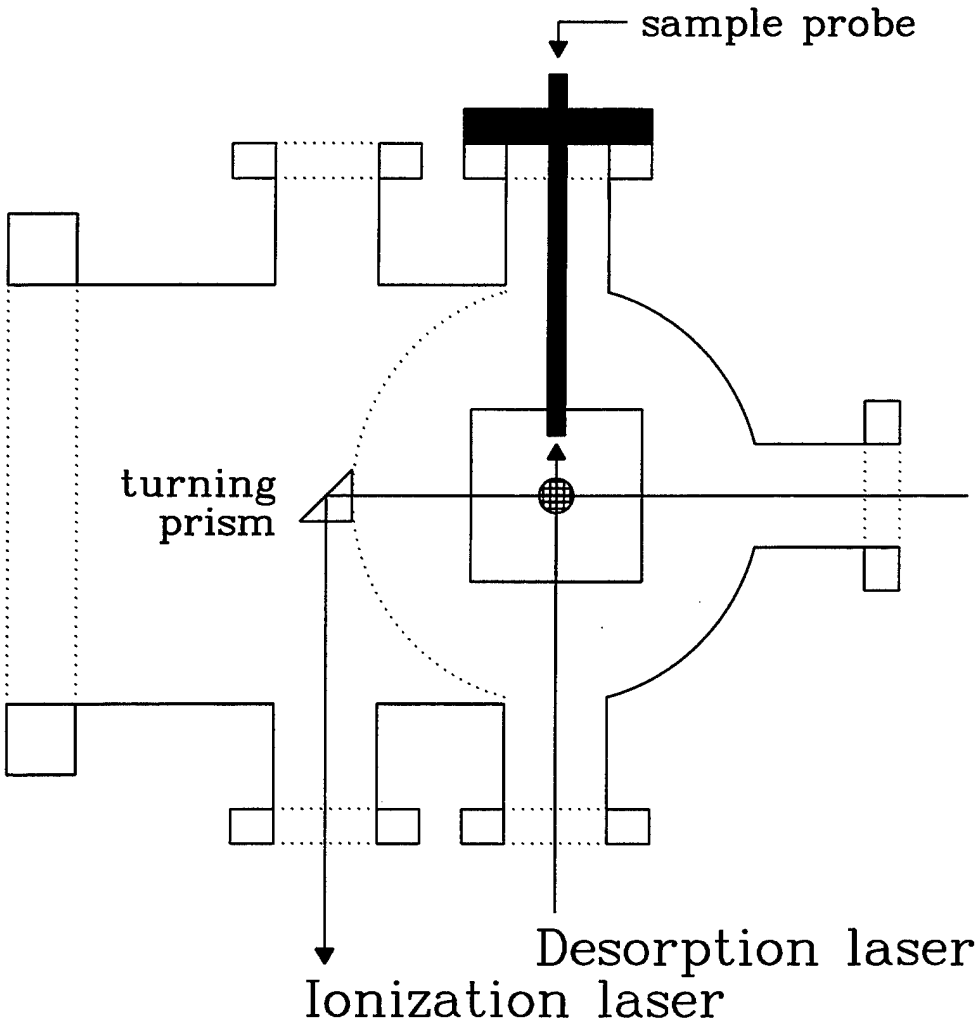

Figure 3: Top view of the LD/LI TOF-MS mass spec chamber In the configuration shown, the desorption laser is normal to the probe surface and the ionization laser crosses the desorbed plume at the center of the ionization region of the mass spectrometer. The ionization laser pulse is passed out of the apparatus by a right angle turning prism mounted inside the vacuum chamber.

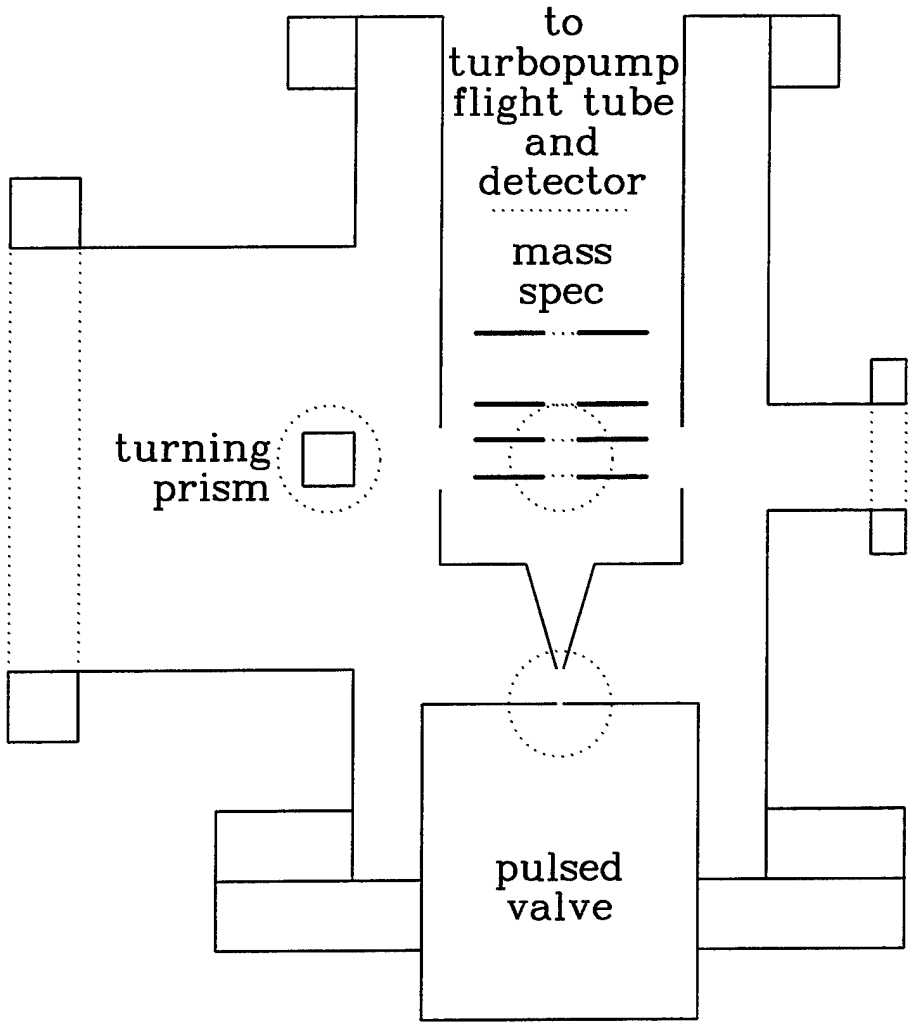

Figure 4: Side view of the LD/LI TOF–MS apparatus showing the lower source chamber which houses the pulsed valve, and the upper mass spec chamber which houses the ion optics and ion detector. The dashed circles indicate ports through which the sample probe or the desorption or ionization laser may enter.

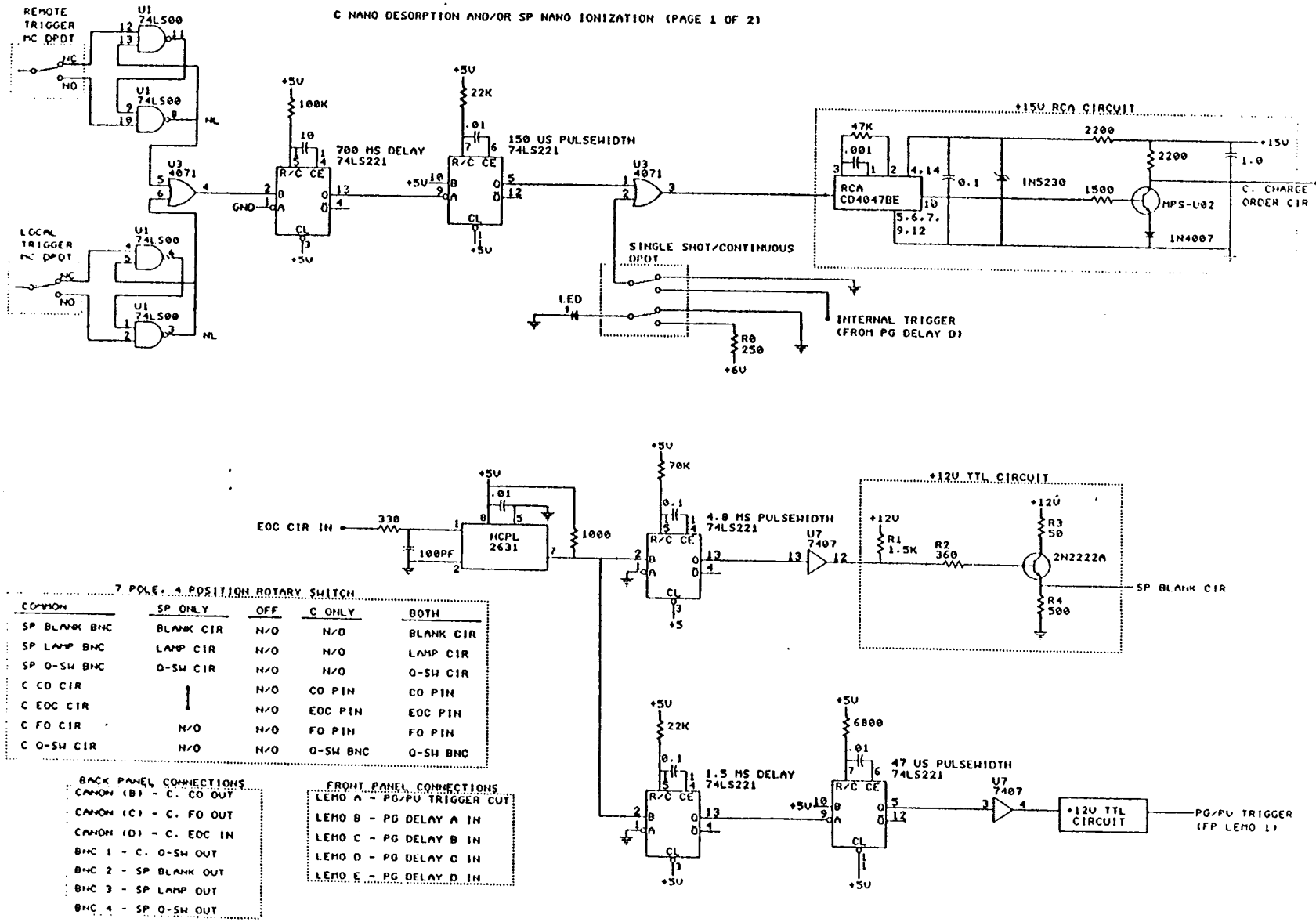

Figure 5: NIM Synchronization Module

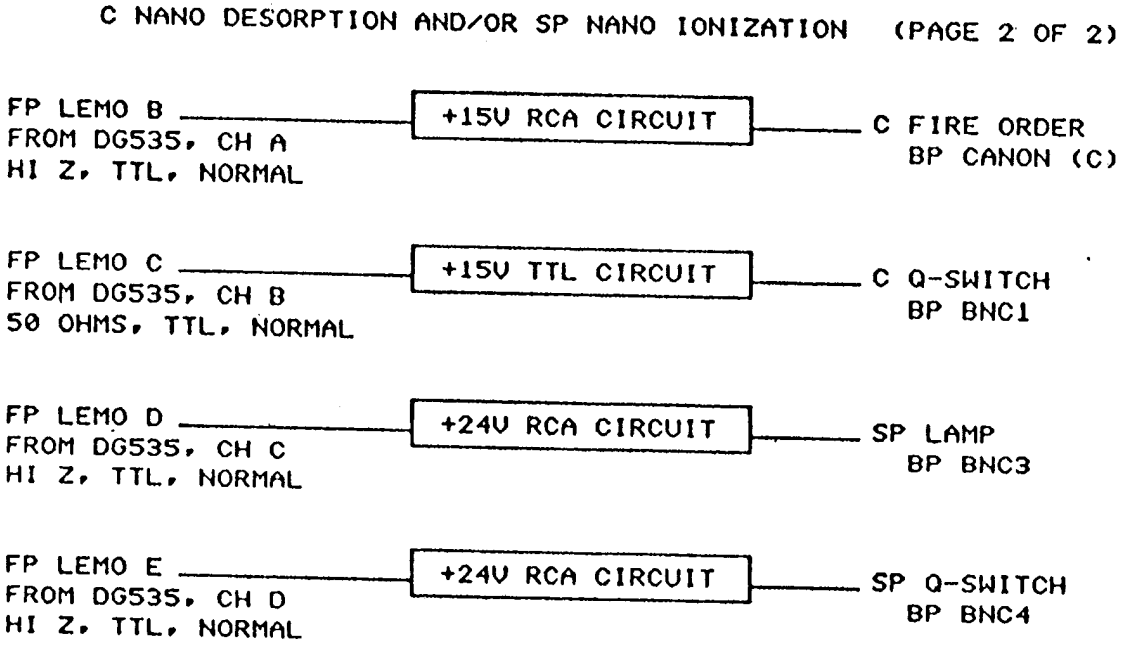

Figure 6: NIM Module (continued)

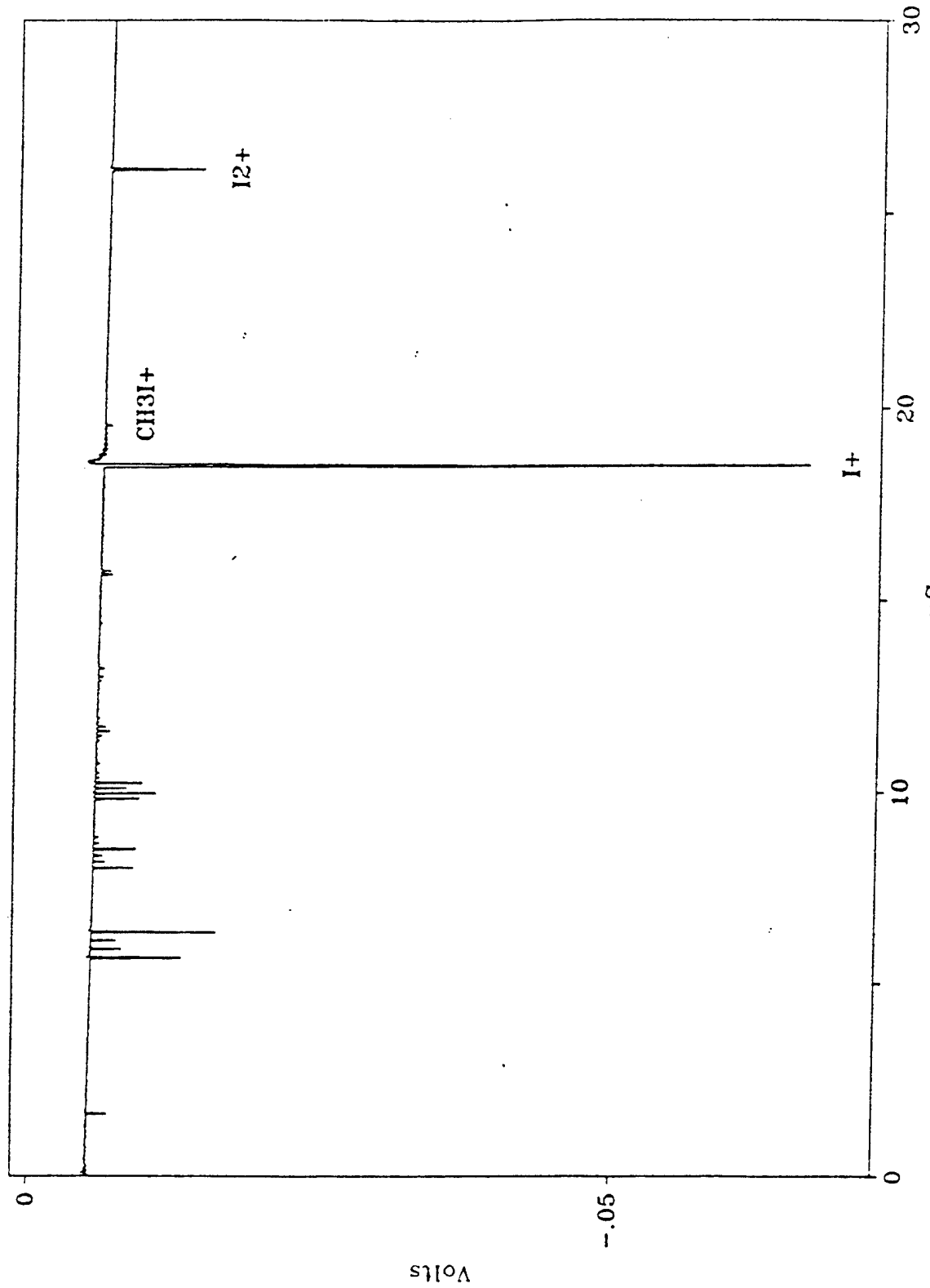

Figure 7: Multiphoton Ionization of Methyl Iodide

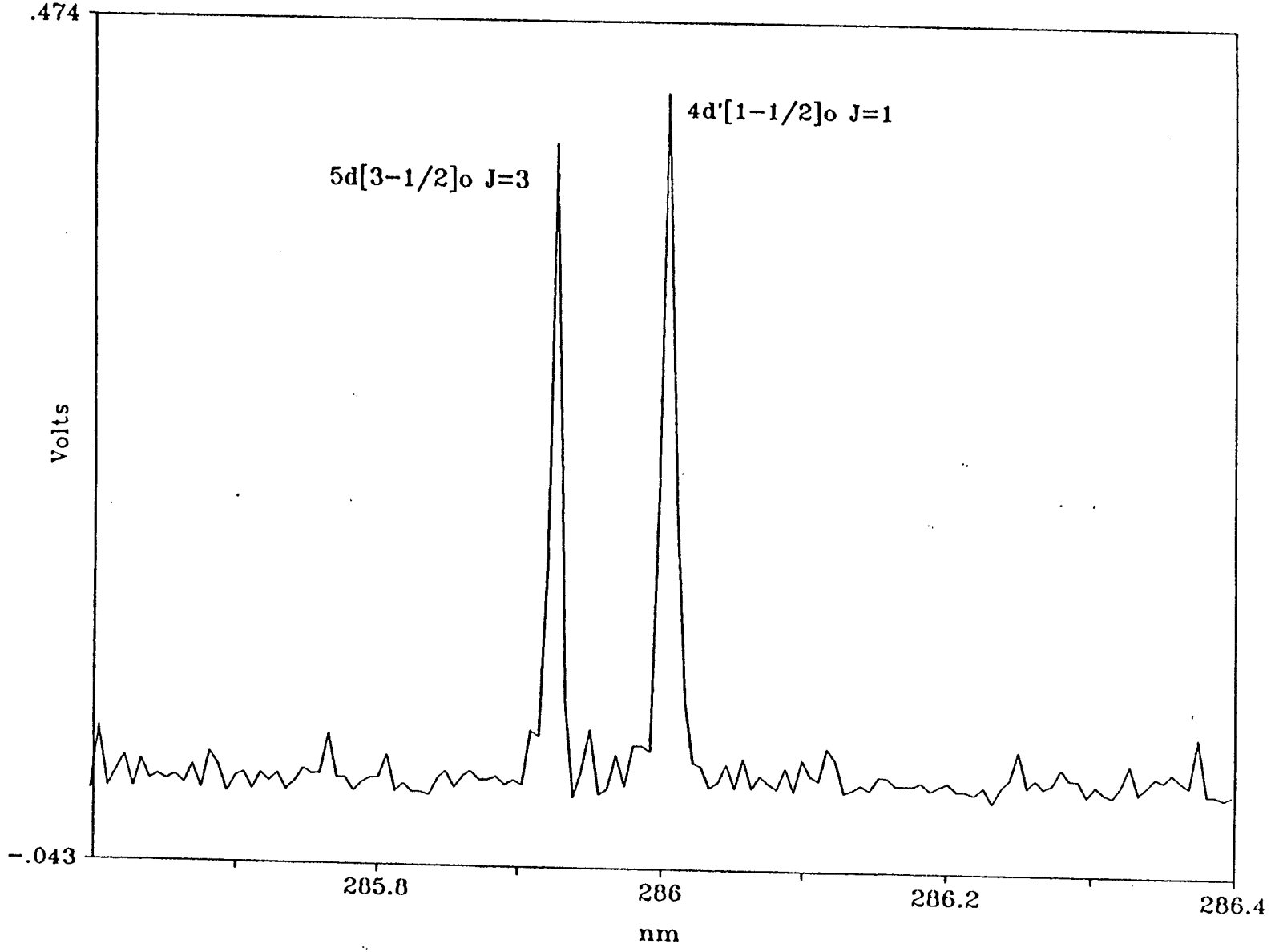

Figure 8: 3+1 Resonance Enhanced Multiphoton Ionization of Krypton

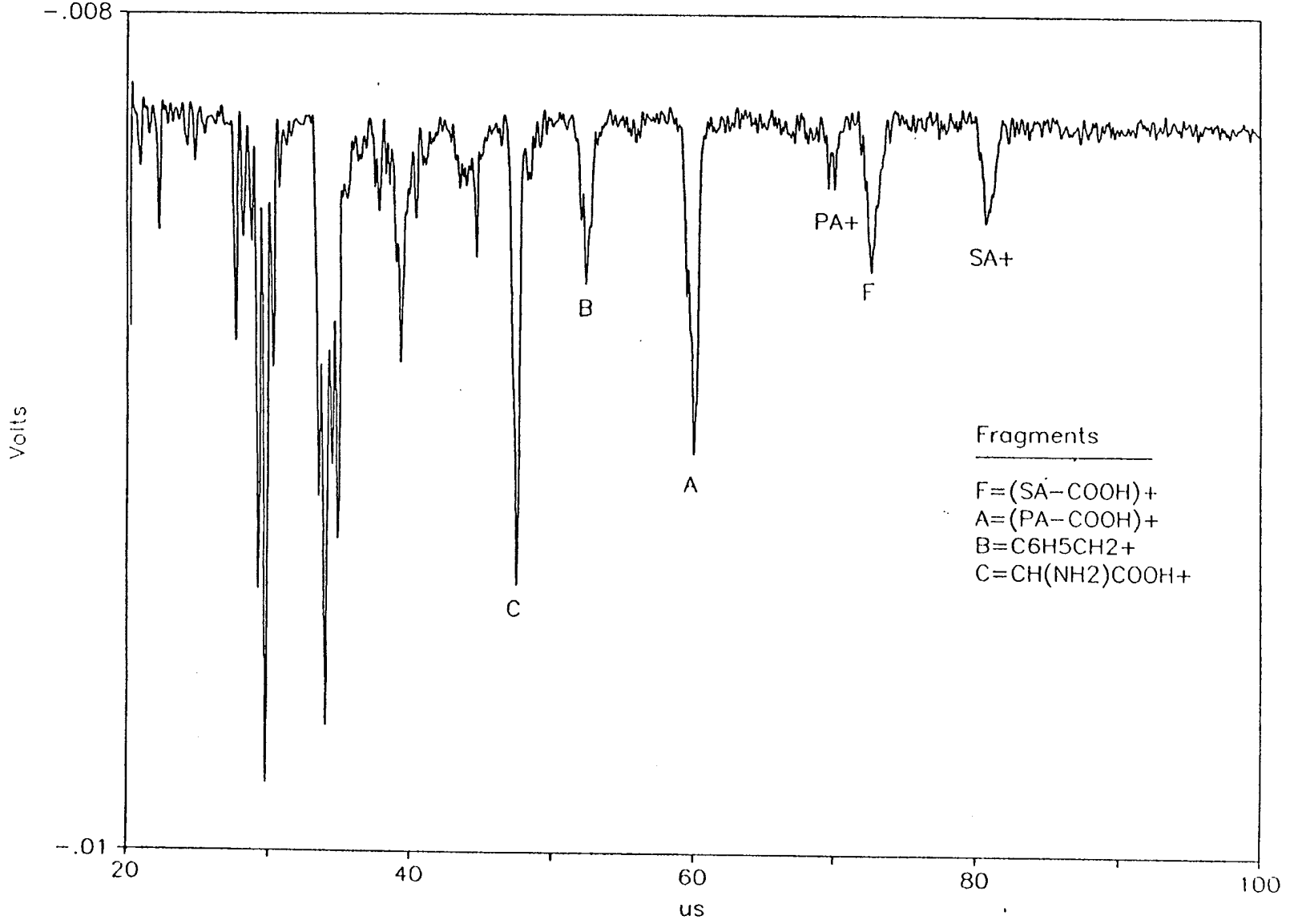

Figure 9: MALD/LI of Phenylalanine in Sinnapinic Acid

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